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The Impact of Weight-bearing Exercise, Non–Weight-bearing Exercise, and Cardiovascular Stress on Biochemical Markers of Cartilage Turnover in Patients With Mild to Moderate Knee Osteoarthritis – A Sequential, Cross-Over, Clinical Study

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Abstract

Objective. To investigate how running, cycling, and sedentary cardiovascular stress impact biomarkers of cartilage turnover acutely in subjects with knee osteoarthritis (OA). *Design.* This was a sequential, cross-over, clinical study. Forty subjects with primary knee OA underwent moderate-to-high-intensity cycling, running, and adrenaline infusion on separate days. Blood was sampled before, during, and at 6-time points after intervention. On a control day, similar samples were taken. Biomarkers of type II collagen degradation (C2M, T2CM, Coll2-1, Coll2-1NO2), formation (PRO-C2), and aggrecan degradation (ARGS) were measured. *Results.* Mean age was 60.4 years, 40% were male, 45% had cumulated Kellgren-Lawrence (KL)-grade (Right + Left knee) of 2 to 3 and 55% had 4 to 6. Analyzing overall changes, area under the curve was significantly lower compared with resting values for ARGS and C2M after cycling and for ARGS after running. Considering individual time points, peak changes in biomarker levels showed reduction in C2M shortly following cycling (T_{20min} = −12.3%, 95% confidence interval [CI]: −19.3% to −5.2%). PRO-C2 increased during cycling (T_{10min} = 14.0%, 95% CI = 4.1% to 23.8%) and running (T_{20min} = 16.5%, 95% CI = 4.3% to 28.6%). T2CM decreased after cycling (T_{50min} = −19.9%, 95% CI = −29.2% to −10.6%), running (T_{50min} = −22.8%, 95% CI = −32.1% to −13.5%), and infusion of adrenaline (peak, T_{50min} = −9.8%, 95% CI = −20.0% to 0.4%). A latent increase was seen in Coll2-1 240 minutes after running (T_{260min} = 21.7%, 95% CI = −1.6% to 45.1%). *Conclusion.* Exercise had an impact on cartilage markers, but it did not suggest any detrimental effect on cartilage. Changes following adrenaline infusion suggest a sympathomimetic influence on the serological composition of biomarkers.

Keywords

knee osteoarthritis, exercise, biochemical markers, type II collagen, adrenaline

Introduction

Exercise influences plasma constituents[1] including markers of joint tissue degradation and formation processes[2] in reflection of biomechanical stress on cartilage turnover or the circulatory stress associated with vigorous exercise. Biochemical markers are investigated for indication of degradation and formation of cartilage and thereby selection of patients and monitoring treatment by, e.g., disease-modifying osteoarthritis drugs (DMOADs) to inhibit cartilage loss and/or promote formation.[3] Several biomarkers reflecting turnover of cartilage collagens and proteoglycans are proposed.[4] Management strategies for knee osteoarthritis (OA) include physical activity,[5] but the impact of exercise on collagen homeostasis is not clear.[6] Various types of exercise influence the joints differently[7] and, in turn, the cartilage. Exercise may elevate

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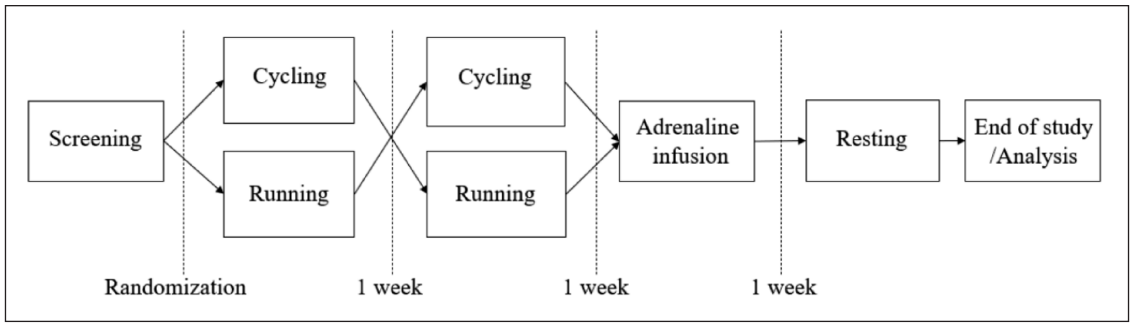

Figure 1. Overview of study design.

biomarkers of type II collagen turnover, CPII, and PIIANP, within 15 minutes in OA patients.[8] Yet, a marker of cross-linked type II collagen turnover, CTX-II, remained unchanged in synovial fluid and urine after knee-extension exercise.[9] Cycling and running may impact both collagenous and non-collagenous biomarkers, without distinction between exercise modality.[2,10] However, in a pilot study, EFEX-OA-01,[2,10] lacked statistical power and suggested a need for methodological improvement including a longer follow-up on intervention days and strict inclusion criteria. Furthermore, the study indicated a need for evaluation of the impact of cardiovascular stress on the biomarker levels, as biomarker changes were detected in response to a presumed gentle type of knee loading—ergometer cycling—which was intended as a cardiovascular control. Therefore, it remains unclear how exercise with different types of stress, impact versus shear, on arthrotic joints affect biomarkers of cartilage turnover. This insight is interesting in the light of the perception of OA as a result of "wear and tear," but more importantly valuable and important in the understanding of the biomarkers in OA and can have potential significance for the OA clinical development. This study included markers of type II collagen degradation (C2M, T2CM, Coll2-1, Coll2-1NO2) and formation (PRO-C2) as well as a marker of aggrecan (ARGS) turnover. We judged that a refined and expanded exploratory study was indicated constituting a reliable follow-up on our described pilot study. Our objective was to explore how weight-bearing versus non–weight-bearing exercise impact biomarkers reflecting cartilage turnover in subjects with knee OA. As a central novel intervention and comparator, we included intravenous adrenaline infusion to mimic the cardiovascular stress of exercise, but without muscle and joint involvement. We hypothesized that cardiovascular stress induced by adrenaline infusion has an impact on circulating biomarker concentrations.

Materials and Methods

Study Design

This study had a randomized, sequential, cross-over design. The short-term effect of cycling, running, and adrenaline infusion versus rest on joint biomarkers in primary knee OA patients was investigated on 4 separate days 1 week apart (**Fig. 1**): Two exercise interventions, one pharmacological exercise simulation, and one resting control visit. Participants underwent cycling and running in a randomized order (by

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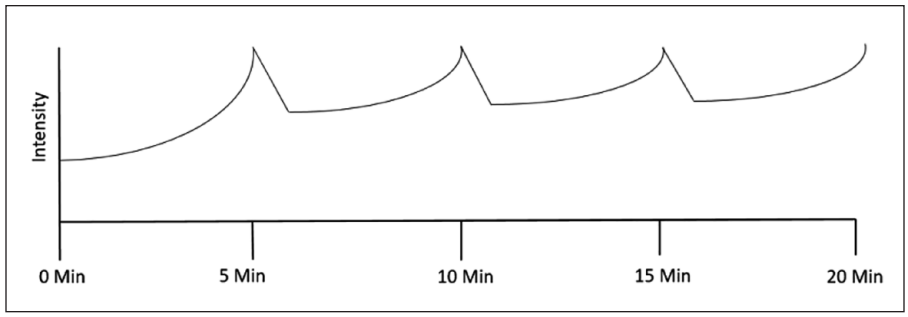

Figure 2. A schematic illustration of the intensity regulation during running, cycling, and exercise simulation.

coin toss), then completed i.v. adrenaline infusion to mimic the effect of exercise on the circulation without skeletal muscle and joint involvement, and a resting visit with similar sampling time points. Participants were instructed to be minimally active on the day before and on the day of intervention (carry out only activity of daily living and to drive or use public transportation). Furthermore, subjects were required to fast for 6 hours before visits and throughout the study day. All visits were initiated with a 30-minute resting period to reduce signals from previsit physical activity.

Participants

The inclusion criteria were 40 to 75 years of age, bodyweight 50 to 100 kg, body mass index (BMI) 18.5 to 35.0 kg/m^2, and primary knee OA with a cumulated Kellgren-Lawrence (KL) radiological grade of at least 2 in the knees, meaning that patients with knee OA due to trauma or inflammatory joint disease including chondrocalcinosis (secondary OA) were not included and that inclusion provided at least KL1 in both knees or KL2 in at least one knee. Exclusion criteria were knee OA KL-grade 4 in one or both knees, history of arthroscopy, or intra-articular injections in the knee within 6 months before screening, previous arthroplasty of knee or hip, and intended major surgery during the timeframe of the study. Also treatment with beta-blockers, monoamine oxidase inhibitor (MAO) inhibitors, systemic corticosteroids, vitamin K antagonists, new oral anticoagulants or heparin, active systemic infection, active systemic inflammatory immune or autoimmune disease, any sign of previous or current cardiovascular disease, or being an active athlete or highly trained individual were exclusion criteria. The study was conducted in accordance with the standards of the Helsinki Declaration and approved by the Regional Ethical Committee (no. H-20026057) and registered at clinicaltrials.org (no. NCT04542668).

Procedures

At baseline, all subjects filled in the knee injury and osteoarthritis outcome score (KOOS) pain questionnaire,[11] had bilateral knee x-ray, electrocardiography, and pulse-oximetry for 5 minutes supine rest to determine resting heart rate (HR). A Siemens Multix Fusion Max radiography machine (Erlangen, Germany) was used with a SynaFlexer device (Bioclinica, Princeton, New Jersey) for consistent knee positioning during radiography, and images were analyzed by an experienced radiologist.

Exercise and infusion sessions included four progressive 5-minute intervals (**Fig. 2**) to reach high intensity during a realistic duration of activity for OA patients, considering the assumed associated pain and discomfort. Cycling was carried out on a Monark Ergomedic 874e ergometer (Stockholm, Sweden) and running on a Masterfit TP100 treadmill (Abilica, Herlev, Denmark) and aimed at >80% of the heart rate reserve (HRR). Maximal heart rate (HR_{max}) was estimated as 206.3 − (0.711*age) beats/min and HRR was HR_{max} − resting HR and monitored by a Polar H10 chest sensor (Kempele, Finland).

For adrenaline infusion, 0.06 mg/kg was prepared in a 50 mL saline solution and administered i.v. using an Infusomat Space pump (B. Braun, Frederiksberg, Denmark). The participants were recumbent and under electrocardiographic monitoring. The flow rate was adjusted by the investigator to mimic the exercise-HR, while only allowing tolerable discomfort.

On days of intervention, blood was drawn at baseline (T_0), once midway during exercise/infusion (T_{10}), immediately after completion (T_{20}), and at 50, 80, 140, 260 minutes and after approximately 24 hours. At rest, samples were collected at similar time points, except for the 24-hour follow-up sample. Samples were collected from sitting participants during and after exercise and on the day of rest. During adrenaline infusion, the first three samples were collected with the participant supine, whereas the additional samples were from participants sitting. All visits were initiated between 8:00 and 12:00 a.m. Any adverse events (AEs) in relation to study procedures were registered.

Biomarkers

Levels of serum C2M,[12] reflecting type II collagen degradation by matrix metalloproteinases (MMPs), T2CM[13]

reflecting type II collagen degradation by MMP-1 and MMP-13 and Coll2-1 and Coll2-1NO2,[14] reflecting type II collagen degradation, and PRO-C2,[15] a propeptide-fragment of type II collagen reflecting type II collagen formation were measured using enzyme-linked immunosorbent assay (ELISA) assays. In addition to the collagen markers, serum ARGS,[10] reflecting aggrecan degradation by ADAMTS-5, was measured using ELISA. Information on lower limit of detection (LLOD), quantifiable range, and intra-assay and inter-assay coefficients of variation (CVs) was obtained from the respective manufacturers.

The C2M and PRO-C2 were run on automated assay platforms targeting mean range ± 25% and 20%, respectively, with 2 quality checks (QC) and no exceptions. The ARGS and T2CM were run manually targeting mean range ± 20% with 1 of 3 QCs allowed ± 20%. For Coll2-1 and Coll2-1NO2, internal controls should lie within a predefined range with no exceptions. Concentrations below the lower limit of quantification (LLOQ) or above the upper limit (ULOQ) were set to the LLOQ and ULOQ levels, respectively, in the statistical analyses.

Table 1. Baseline Characteristics for Study Subjects.

Baseline parameter	*N* = 40
Mean age, years (SD)	60.4 (8.7)
Male sex, n (%)	16 (40)
BMI, kg/m^2 (SD)	26.9 (3.5)
KOOS pain (SD)	67.5 (15.2)
Cumulated KL-grade, n (%)	KL 2: 6 (15)
	KL 3: 12 (30)
	KL 4: 15 (37.5)
	KL 5: 4 (10)
	KL 6: 3 (7.5)
C2M, ng/mL (SD)	22.5 (8.9)
PRO-C2, ng/mL (SD)	21.5 (15.1)
T2CM, ng/mL (SD)	7.9 (8.8)
Coll2-1, nmol/mL (SD)	670.0 (162.3)
Coll2-1NO, pg/mL (SD)	1,093.9 (865.3)
ARGS, pmol/L (SD)	78.7 (115.7)

R = range; SD = standard deviation; BMI = body mass index; KL-grade = Kellgren-Lawrence grade; cumulated KL-grade = KL grade of the left + right knee.

Statistical Analyses

As this was an exploratory study, no formal power calculations were performed. In this report, *P*-values < 0.05 are highlighted as significant and no adjustment for multiple testing was performed. Changes in biomarker concentrations after activity were compared with both the corresponding resting samples and to baseline. For overall changes during 260 minutes following cycling, running, and adrenaline infusion, we used analysis of covariance (ANCOVA) and Dunnett's test with geometric means of change in area under the curve (AUC) for $T_{0\text{-}260}$ as the dependent variable and subject and activity as covariates. In order to assess changes in biomarkers over time, we used ANCOVA and Dunnett's test with geometric means of change from baseline to 260 minutes as the dependent variable and subject and activity as covariates. Paired *t* test was used to compare values at 24 hours to baseline. Interday variability of each biomarker was calculated as an interclass correlation between baseline values from each of the four study visits. Statistical analyses were carried out using SAS version 9.4, and figures were produced using GraphPad Prism 9.1.0.

Results

A total of 59 subjects were screened of which 19 did not fulfill the inclusion and/or fulfilled one or more of criteria for exclusion. For the included patients, their mean age was 60.4 years (standard deviation [SD] = 8.7), 16 (40%) were male, mean BMI was 26.9 kg/m^2 (SD = 3.5), 18 had cumulated KL-grade of 2 or 3 (45%) and 22 (55%) had cumulated KL 4, 5, or 6, and mean KOOS pain at baseline was 67.5 (SD = 15.2) corresponding to mild-to-moderate pain (**Table 1**).

Only 39 subjects completed cycling as one lacked limb mobility due to a past bone fracture, 37 completed running as three developed intolerable pain, and 35 completed infusion as one did not attend for infusion study visit and in four subjects, intravenous access could not be established. For the study at rest also, one subject did not show up (**Fig. 3**). All subjects reached the minimum peak HR or higher during exercise, but reached only on an average 70% (SD = 8.7) of the targeted HR during adrenaline infusions. No cross-over effects were detected in the statistical analysis, indicating no influence of the sequence of running and cycling.

Acute Changes in Biomarkers Relative to Baseline at Individual Time Points

Cycling induced a small reduction in C2M between T_{20}-T_{50} peaking at −6.8%, whereas T2CM decreased after both cycling (peak: −10.8%, T_{10}-T_{50}) and running (peak: −9.5%, T_{10}-T_{50}) to increase in the recovery peaking at T_{260} after cycling (9.6%) and T_{140} after running (5.9%). A −9.6% change in T2CM was also detected during adrenaline infusion ($T_{10\text{-}20}$) and a transient increase was detected in the resting study (peak: 13.3%, $T_{50\text{-}80}$). A latent increase of 21.3% was found in Coll2-1 in response to running at T_{260}. A transient increase in Coll2-1NO2 was induced by both cycling (peak: 12.5%, $T_{10\text{-}20}$) and running (9.8%, T_{20}). The PRO-C2 increased 11.7% at T_{10} and also 12.9% in response

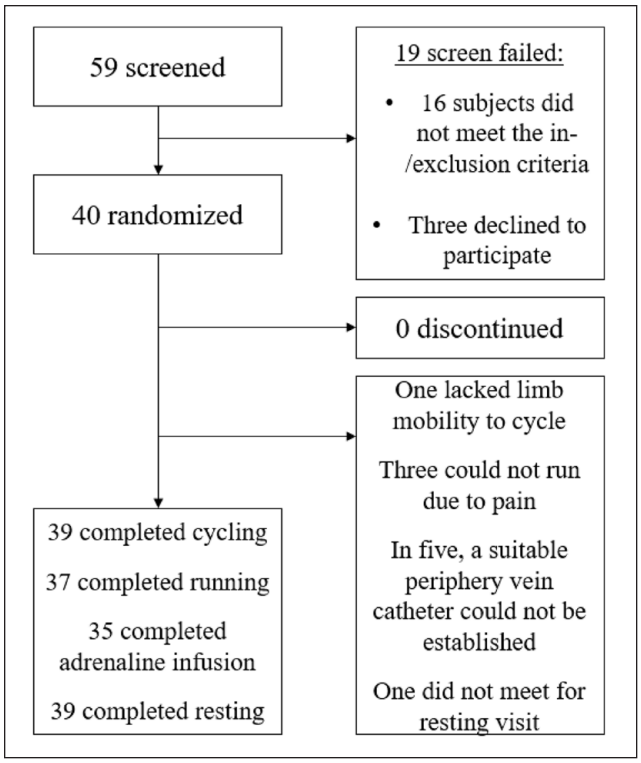

Figure 3. Consolidated Standards of Reporting Trials (CONSORT) chart displaying screening to enrollment.

to running at $T_{10\text{-}20}$. A slight transient decrease to −5.3%, in ARGS was detected in relation to cycling (T_{10}-T_{20}) and again during recovery (peak: −3.5%, $T_{140\text{-}260}$). The ARGS changed −4.6% after running at $T_{80\text{-}260}$, whereas ARGS increased up to 4.1% in response to rest ($T_{140\text{-}260}$). Percentual changes with standard deviation for all biomarkers, interventions, and time points are found in Supplementary Material A (see **Fig. 4**).

Subacute Changes Relative to Baseline (24-Hour Follow-up)

At the 24-hour follow-up, C2M and Coll2-1 were not significantly different from baseline. T2CM was elevated by 6.0% after running, by 7.1% after cycling, and by 4.7% after adrenaline infusion. Coll2-1NO2 decreased after running by −8.3% and PRO-C2 returned to baseline after running and adrenaline infusion, but was reduced −9.4% after cycling. Finally, ARGS decreased −4.1% after cycling.

Acute Changes Relative to Resting Values

None of the AUCs between T_0 and T_{260} for the biomarkers were increased compared to rest. The C2M decreased in response to cycling ($P < 0.05$) and ARGS decreased in response to running and cycling ($P < 0.0001$). The comparison of AUCs following cycling, running, and adrenaline versus rest is displayed in **Figure 5.**

We calculated the difference between the relative changes at each time point at rest and the corresponding time points during cycling, running, and adrenaline infusion. C2M was stable to the impact of running and adrenaline, whereas it was changed −12.3% immediately after cycling (T_{20}). The T2CM decreased up to 30 minutes (T_{50}) after the cycling bout and running peaking at −19.9% and −22.8%, respectively.

A 21.7% increase in Coll2-1 was observed at T_{260} after running. Coll2-1NO2 increased during both cycling (15.8%) and running (15.2%) and then returned toward resting levels (from T_{50} and onwards). An immediate increase in PRO-C2 was found in response to both cycling (T_{10}: 14.0%) and running ($T_{10\text{-}20}$ peak: 16.5%). ARGS decreased transiently during cycling (−6.6%) and again at T_{140}-T_{260} thereafter (peak: −6.9%). After running ARGS changed −7.2% at $T_{80\text{-}260}$. Percentual changes relative to the corresponding resting value for all biomarkers, interventions, and time points are found in Supplementary Material B.

Adverse Events

No AEs occurred in relation to cycling or running. In relation to adrenaline administration, two AEs occurred: One subject experienced unilateral pale fingers and one general tremor that resolved immediately after the infusion and the pale fingers were treated with a warm hand bath after the infusion.

Technical Performance of Assays

The interday variability for each biomarker is reported as interclass correlation coefficients (ICCs) in **Table 2** along with technical performance specifications. The ICC is calculated based on baseline values obtained on each of the four intervention days. Interday variability was very low for T2CM and ARGS (ICC = 0.98), low for PRO-C2 and Coll2-1NO2 (ICC >0.9), moderate for C2M (ICC = 0.76), and high for Coll2-1 (ICC = 0.23).

Discussion

In this study, we explored the impact of running and cycling on biomarkers reflecting cartilage turnover and tested the hypothesis that adrenaline infusion would impact circulating biomarker concentrations. Adrenaline infusion was used to mimic the exercise-induced cardiovascular response without joint or muscle involvement. The main findings are: Cycling and running were observed to decrease serum biomarkers indicative of type II collagen turnover, and similarly, a decrease in these biomarkers was noted following adrenaline infusion alone.

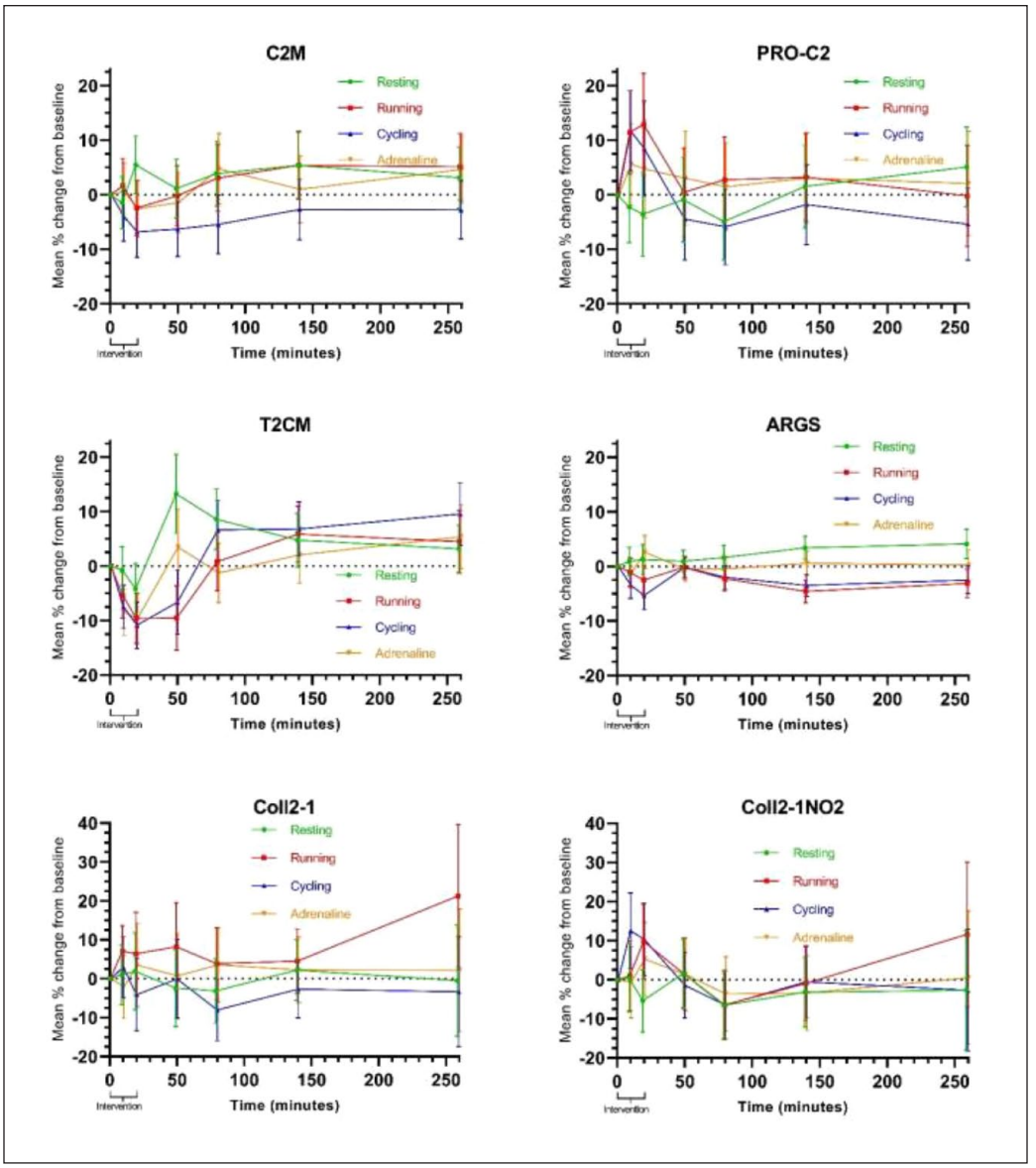

Figure 4. Biomarker dynamics. Error bars represent 95% confidence intervals.

Generally, the impact of moderate-to-high-intensity exercise regardless of load on the knees was low to moderate across four markers of type II collagen degradation as expected based on our pilot study,[2,10] but the direction of change differed between biomarkers and part of the change in biomarker concentrations may be attributable to the cardiovascular stress associated with exercise. We did not find an increase in markers of type II collagen or aggrecan degradation during or 4 hours after non–weight-bearing or weight-bearing exercise, except for Coll2-1, which increased after running at the final 4-hour sampling point. Thus, the data did not indicate acute wear-and-tear.

Conversely, two markers of type II collagen degradation were reduced after exercise of which one also decreased during infusion of adrenaline. Although T2CM also decreased during and shortly after cycling and running, the increase was seen at the later time points, possibly reflecting increase in MMP-mediated type II collagen with latent degradation. PRO-C2, indicative of type II collagen formation, increased ~10% during and after running and cycling and was reduced by ~10% at 24 hours, suggesting a difference in the cartilage repair response between exercise modalities. For aggrecan, a small biphasic reduction was found in ARGS. Aggrecan is suggested as more prone to degradation by small changes in joint homeostasis,[16] but the current results do not indicate increased degradation of aggrecan due to exercise regardless of mechanical impact.

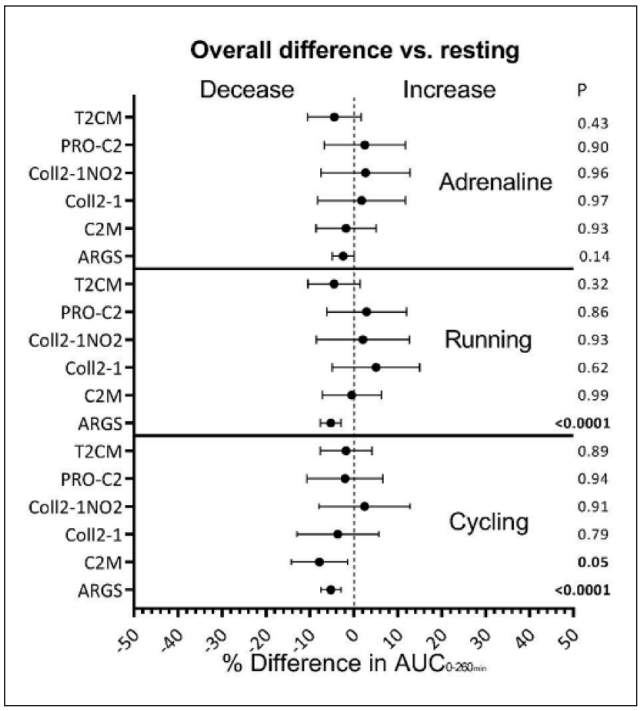

Figure 5. Overall difference in biomarker changes versus resting. Changes are based on comparison of the geometric means area under the curve (AUC).

Although several studies have investigated long-term impact of exercise on biochemical markers related to OA, only few studies have investigated the acute impact and with regard on cartilage oligomeric matrix protein (COMP). Thus, the literature of impact of short-term exercise on type II collagen is sparse.[6] In OA subjects, exercise does not change CTX-II, a marker of cross-linked type II collagen turnover.[2,9,17] Although associated with structural severity and progression,[18,19] CTX-II is a urine-based marker and thus has limited use as a marker of acute changes.

In past, a previous study a serum marker of type I and II collagen degradation, C1,2C, increased 5.5 hours after a 30-minute walk compared with the reference sample taken at 0.5 hours,[20] indicating a delayed effect leading to increased bone and cartilage turnover. C2M, reflecting MMP-mediated type II collagen degradation, increased in response to 30 minutes cycling, but not running, and PRO-C2, reflecting type II collagen formation, increased in response to running.[2] Thus, this study reproduced an increase in PRO-C2 in response to exercise, whereas exercise-evoked increase in C2M was not confirmed. Conversely, C2M decreased in response to cycling, which may be explained by the initial resting period in this study, possibly dampening signals of pre-visit physical activity.

In young healthy subjects, PRO-C2 levels are not affected by exercise.[21] Considering that new formation of type II collagen in cartilage is thought to be limited or non-existent,[22] the increase in PRO-C2 in response to mechanical stimuli of arthrotic joints could reflect type II collagen repair rather than formation. T2CM has previously been found to be upregulated in severe OA.[13] Our results suggest a biphasic response in T2CM to exercise, with decrease during and shortly after exercise followed by an increase, possibly reflecting slightly elevated type II collagen degradation. Coll2-1 and Coll2-1NO2, which have previously been found to be associated with OA,[14,23,24] were, in a particular study, found not to change in response to a marathon run in middle-aged runners.[25] Our findings suggest that Coll2-1 increases after 4 hours possibly reflecting delayed degradation of type II collagen via different mechanisms than those leading to acute turnover. Previous research indicates that release of ARGS could increase in response to exercise.[10] However, in this study, ARGS was stable and may decrease slightly in response to exercise, which is in concert with a decrease in serum aggrecan following exercise,[17] suggesting that exercise does not induce acute aggrecanase-mediated aggrecan breakdown.

The magnitude of the observed changes in biomarkers was generally small. The potential causes of this observation are multiple, although the available evidence does not allow for conclusions on this point. It is possible that the magnitude of joint protein changes either did not occur fast enough to lead to measurable changes in the associated biomarkers within the measurement period, meaning that the exercise/cardiovascular challenges are not sufficient to induce the desired changes in joint tissue turnover, or that any released biomarker protein fragments did not reach the systemic circulation within the measurement period. From a hypothetical clinical perspective, the data might suggest that brief periods of high intensity exercise do not negatively or positively affect joint structure in people with OA of the knee, although this would require further adequately designed experiments to fully conclude.

The Model

Previously, a clinical model was proposed to investigate the acute impact of exercise on joint tissue turnover as reflected in biochemical markers.[2] This model was further developed in this study aiming at generating clearer results more accurately describing ongoing biological processes. Improvements included more subjects, higher, multiphasic exercise intensity, an initial period of rest, additional sampling points, prolonged sampling period, and adrenaline infusion. These improvements in the design may explain the discrepancy between results of past studies and those presented here. Furthermore, new markers were added, whereas others were deselected based on the previous results.[2] Changes in PRO-C2 and T2CM as well as latent

Table 2. Technical Performance of the Individual Biomarkers.

Biomarker	Quantifiable range / measurement range	Intra-assay CV	Inter-assay CV	Interday variability
sC2M	0.13-2.48 ng/mL	6.6%	11.6%	0.76 (0.63-0.86)
sPro-C2	11.96-1,078.37 ng/mL	3.8%	8.4 %	0.93 (0.88-0.96)
T2CM	1.20-93.85 ng/mL	4.0%	13.0%	0.98 (0.97-0.99)
ARGS	37.0-684.0 ng/mL	4.7-6.9%	1.4-6.2%	0.98 (0.96-0.99)
Coll2-1	31.25-2,000 nM	8.2%	9.3%	0.25 (0.08-0.45)
Coll2-1NO2	39-2,500 pg/mL	6.9%	9.9%	0.91 (0.85-0.95)

Information on lower limit of detection (LLOD), quantifiable range, intra-assay coefficient of variance (CV), and inter-assay CV was provided by the respective assay manufacturers. Interday variability was calculated as the intraclass coefficient between baseline values at the four intervention visits.

impact of exercise on Coll2-1 and Coll2-1NO2 warrant investigation beyond 4 hours.

Limitations

The biomarker changes observed during and immediately after exercise and adrenaline infusion should be interpreted with caution, as they may reflect a wash-out effect due to the increase blood flow in the joint tissue or be due to transient exercise-induced hemoconcentration (~10%).[26] The follow-up time with no samples between 4 and 24 hours post-exercise limits identification of the complete biomarker kinetics. The post-exercise sampling time was extended by 1.5 hours compared to the pilot study[2] to detect full trajectories. However, Coll2-1 and Coll2-1NO2 were the highest at the final time point (T_{260}) suggesting potential for a further increase. It is also a limitation that patients could have OA in other joints involved, limiting the relationship between the degree of OA in the patients' knees and the changes in biomarkers. Adrenaline infusion was intended to mimic circulatory stress from exercise without joint involvement but induced a slightly lower HR than exercise and likely limited inotropic effect because the rate was chosen to be well tolerated. However, adrenaline had impact on several biomarkers similar to that induced by exercise, but the impact may be slightly lower than if HR had reached that obtained during physical exercise. Furthermore, an age-matched non-OA control group could be considered to detect any causal link between OA and changes in biomarkers. However, this study was designed for subjects to be their own controls, and control visits were included to distinguish contributions attributable to diurnal variation and cardiovascular stress during cycling and running.

Perspectives

Biomechanical stress induced by common exercise modalities, such as cycling and running, does not elicit systemic biochemical signals indicative of joint tissue turnover, specifically collagen II and aggrecan, thereby challenging the notion that OA merely stems from wear and tear.

In terms of the clinical relevance of the study, the study was designed to detect differences in biomarkers of joint tissue turnover and not to describe the safety or other relevant impact on joint health. Hence, the overall conclusions of the study are limited to describing the observed biomarker data and what that could mean from a biochemical and structural perspective and not from a clinical perspective.

Exercise is already considered a cornerstone in overall metabolic and cardiovascular health in OA patients, and although the findings of this study are exploratory, and thus preliminary, we find no clear biomarker signal of negative impact of running or cycling on the cartilage homeostasis, suggesting that it may be subordinate for cartilage health whether patients prefer cycling or running. The feasibility of intravenous adrenaline as a means of inducing circulatory stress without joint and muscle involvement is demonstrated, but other vasoactive drugs with a stronger inotropic profile could be considered. An important perspective for the current model is identification of structural progressors which could enrich clinical development, although this remains hypothetical. Thus, association between changes in biomarkers in response to exercise and subsequent long-term structural progression is reported.[20,27] Also, as proposed by Cattano et al.,[28] biomarker response to loaded exercise could guide return to sport after injury.

Conclusion

In knee OA patients, cycling and running induced small to moderate acute changes in serum biochemical markers of type II collagen degradation and formation as well as aggrecan degradation generally suggesting no detrimental effect on cartilage tissues, but warranting further investigation. Adrenaline infusion, mimicking exercise without muscle and joint involvement, induced changes in biomarkers similar to those observed after exercise, but smaller in magnitude, confirming our hypothesis and suggesting that part of

the changes in circulating biomarkers induced by exercise is attributable to cardiovascular stress and not biomechanical stress to the joints.

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Author Contributions

J.J.B.-B., A.R.B., N.H.S., H.B.N., and P.K. designed the protocol. J.J.B.-B., C.S., M.B., N.H.S., M.U., and C.-C.K. acquired the data. J.J.B.-B., G.D., and V.F. conducted data analyses. J.J.B.-B., Y.H., M.U., C.S.T., and A.R.B. interpreted the data. J.J.B.-B., G.D., V.F., and A.R.B, drafted the manuscript. Critical revision and approval of the manuscript were done by all authors.

Declaration of Conflicting Interests

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Ethics Approval

The study was conducted in accordance with the standards of the Helsinki Declaration and approved by the Regional Ethical Committee (approval no. H-20026057).

Patient Consent

All patients received thorough written and oral study information and consent from all participants was initially secured.

Clinical Trial Registration

Clinicaltrials.org registration no. NCT04542668.

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Data Availability Statement

Research data are not shared.

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